GROWTH RESPONSES OF UNICELLULAR ALGAE TO POLYCHLORINATED BIPHENYLS : NEW EVIDENCE FOR PHOTOSYNTHETIC INHIBITION

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ABSTRACT

The unicellular green algae: *Chlorella vulgaris*, *C. fusca* var. *vacuolata*, *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* show increased resistance to 200, 1, 0.5 and 4 mgl^{-1} (ppm) of a polychlorinated biphenyl (Aroclor 1242) respectively when their culture media contains a heterotrophically available carbon source. In contrast, there is total inhibition of growth when they are cultured photoautotrophically at these levels of polychlorinated biphenyls (PCB's). From the growth patterns observed, it is proposed that these algae do not photosynthesize at these PCB concentrations, but derive their total nutrition heterotrophically from the supplied carbohydrate, thereby by-passing a photosynthetic block caused by PCB's. A critical review of the literature is presented on the effects of PCB's on photosynthesis; some experimental work is questioned on technical grounds. With the facts revealed by this investigation and evidence accumulated from the literature, it is concluded that the primary inhibition induced by PCB's in phytoplankton is responsible for a block in some part of the photosynthetic process.

INTRODUCTION

Polychlorinated biphenyls (PCB's) are an important group of industrial chemicals commonly used in electrical insulators, plasticizers, high pressure hydraulic fluids, heat transfer agents, lubricants and waxes (Fishbein 1972). Although commercially manufactured since 1929, they were not detected as biological contaminants until 1966 when Jenson identified PCB residues in fish, eagle feathers and human hair (Anonymous 1966). Subsequent to this finding, numerous studies consistently revealed PCB's in biotic and abiotic systems throughout the world, including the Arctic and Antarctica, leading to the conclusion that PCB's are ubiquitously distributed throughout the environment.

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Phytoplankton form the basis of many aquatic food chains and consequently many scientists have studied the effects of PCB's on these organisms. Numerous studies have shown that the tolerance of phytoplankton to PCB's varies considerably between species and that some PCB compounds are more toxic than others to a given species (e.g. see Craigie and Hutzinger 1975). The interspecific differences in susceptibility of phytoplankton to PCB's can, in some instances, be related to the relative extent of PCB uptake by the individual species (Harding and Phillips 1978b). The sensitivity of phytoplankton to PCB's has been shown to vary with temperature (Fisher and Wurster 1973), geographic location (Fisher *et al.* 1973), interspecific competition (Mosser *et al.* 1972, Fisher *et al.* 1974) cell density (Cole and Plapp 1974, Harding and Phillips 1978a), nutrient availability (Fisher *et al.* 1976), osmotic pressure (Fisher 1977) and the presence of other toxicants (Mosser *et al.* 1974). It therefore follows that stress and suboptimal conditions may increase phytoplankton sensitivity to PCB's. In view of such differential responses, it is to be expected that PCB's will alter species composition of natural phytoplankton communities, and this has been reported frequently (Fisher *et al.* 1974, Moore and Harriss 1974, Kricher and Bayer 1977, Biggs *et al.* 1978, O'Connors *et al.* 1978). Consequently the health, abundance and distribution of selective phytoplankton-feeders and other animals higher in the food chain could be indirectly affected by PCB's (Wood 1965). Laboratory experiments have shown that PCB's can be taken up from the growth medium and accumulated to high concentration by many phytoplankton species. These include diatoms (Keil *et al.* 1971, Fisher *et al.* 1976, Harding and Phillips 1978b), unicellular green algae (Södergren 1971, 1973, Morgan 1972, Urey *et al.* 1976, Scura and Theilacker 1977), chrysophyes, dinoflagellates and haptophytes (Harding and Phillips 1978b).

Accumulations of PCB's in phytoplankton communities have been reported frequently (Jenson *et al.* 1972, Giam *et al.* 1973, Linko *et al.* 1974, Särkkä *et al.* 1978). Such accumulations must magnify the uptake of PCB's by phytoplankton-feeders and therefore have the potential to directly affect the activity of these animals.

There has been much speculation on the exact nature of the growth inhibition of phytoplankton caused by PCB's. Nevertheless, there is general agreement that the reported growth inhibitions are symptomatic expressions of disruptions in phytoplankton physiology. The most frequently reported physiological effect of PCB's on phytoplankton is a reduction in ^{14}C-bicarbonate uptake (Morgan 1972, Moore and Harriss 1972, 1974, Luard 1973, Cole and Plapp 1974, Fisher 1975, Glooschenko and Glooschenko 1975, Hawes *et al.*1976, Harding 1976, Kricher and Bayer 1977, Powers *et al.* 1977, Biggs *et al.* 1978, Bryan and Olafsson 1978, Harding and Phillips 1978a) which suggests an inhibition of the photosynthetic process. However, Fisher (1975) correctly pointed out that such reductions may also result from fewer cells photosynthesizing, which may be due to PCB's having some other effect on phytoplankton. To overcome this problem it has become standard practice to correct for cell density. Fisher (1975) calculated that PCB's have no significant effect on ^{14}C-bicarbonate uptake per cell in *Thalassiosira pseudonana*, whilst Glooschenko and Glooschenko (1975) reported slight stiumulations in both *Ankistrodesmus falcatus* and *Navicula pelliculosa*. Such

results suggest that PCB's do not have a direct effect on photosynthesis. However, other workers have calculated significant reductions in the ^{14}C-bicarbonate uptake per cell in *Chlamydomonas reinhardtii* (Morgan 1972), *Euglena gracilis* (Ewald *et al.* 1976), *Thalassiosira pseudonna, Chaetoceros socialis, Skeletonema costatum, Monochrysis lutheri, Isochrysis galbana* (Harding and Phillips 1978a) and a marine phytoplankton community (Powers *et al.* 1977), while Hawes *et al.* (1976b) note both reduction and no effect in *Chlorella pyrenoidosa* for different PCB's. Similarly, calculations using chlorophyll content as in index of cell density are also inconsistent. Reduced ^{14}C-bicarbonate uptake per unit of chlorophyll *a* is known from natural phytoplankton communities (Powers *et al.*1977, Biggs *et al.* 1978), however Sinclair *et al.* (1977) recorded slight stimulations in *Chlorella vulgaris* and also that PCB's failed to effect fluorescence, O_2 evolution in flashing light and the Emerson enhancement phenomenon; parameters that are directly or indirectly associated with photosynthesis. Contrary to their conclusion that photosynthesis is not affected by PCB's in *Chlorella vulgaris*, Sinclair *et al.* (1977) presented data from similar experiments on isolated spinach (*Spinacia oleracea*) chlorplasts, suggesting that some part of the electron transport chain associated with photosystem II contains a site sensitive to PCB's.

The hypothesis that PCB's disrupt chloroplast function is substantiated by electron microscopy studies showing distortion of internal chloroplast membranes compared with little or no effect on other cell components in the aquatic angiosperm, *Spirodela oligorrhiza* (Mahanty and Fineran, 1976). Glooschenko and Glooschenko (1975) briefly commented on similar disruptions of chloroplast thylakoids and increased vacuole formation in several unicellular algae. In addition, PCB's have been reported to inhibit 0_2 evolution in both *Scenedesmus obtusiusculus* (Larsson and Tillberg 1975) and *Chlorella vulgaris* (Sinclair *et al.* 1977), *in vivo* chlorophyll *a* fluorescence in *Ditylum brightwellii* and *Lauderia borealis* (Harding and Phillips 1978c) and to decrease chlorophyll levels in the marine diatom, *Cylindrotheca closterium* (Keil *et al.* 1971), the freshwater flagellate, *Euglena gracilis* (Ewald *et al.* 1976), a marine phytoplankton community (Powers *et al.* 1977)and the higher plant, *Spirodela oligorrhiza* (Mahanty and McWha 1976).

Thus present knowledge concerning the effects of PCB's on photosynthesis is in a state of considerable confusion. As similar chlorinated hydrocarbons, such as DDT and DDE, are known to inhibit the light reaction of isolated chloroplasts from the unicellular green alga *Dunaliella tertiolecta* (Bowes 1972) and a variety of other plants (Lawler and Rodgers,1967,1968, Bowes and Gee 1971), this investigation was designed to re-examine the effects of PCB's on the photosynthesis of phytoplankton. In doing so, it was necessary to design a completely new approach, rather than add to the confusion that already exists from ^{14}C-bicarbonate experiments. Therefore a simple, yet novel procedure was employed. This involved monitoring the growth of unicellular algae when they were cultured heterotrophically and photoautotrophically in the presence and absence of PCB's, to examine for any differences in growth patterns. In other words, are unicellular algae more resistant to PCB's when they are not photosynthesizing, but still actively growing and reproducing through heterotrophic nutrition?

MATERIALS AND METHODS

ALGAL STRAINS AND GROWTH MEDIA

Axenic cultures of the following freshwater unicellular algae were used in this study: *Chlorella vulgaris* (strain 211/8k) and *C. fusca* var. *vacuolata* (strain 211/8b) from the Cambridge Culture Collection, as well as *Scenedesmus obliquus* (strain UTEX 2015) and *Chlamydomonas reinhardtii* (strains UTEX 89+ and UTEX 90-) from the University of Texas Culture Collection.

Each of these strains grow well in the liquid TAP (Tris-acetate-phosphate) medium of Gresshoff (1976). A minimal form of this medium lacking a carbon source (MTAP) was used and consisted of: 50 ml of 0.78M potassium phosphate buffer (pH 6.5), 50 ml of Beijerinck's solution, 1 ml of a trace element solution and distilled water to 1000 ml. Beijerinck's solution and the trace element solution were prepared as described by Gresshoff (1976). When mixotrophic or heterotrophic growth of the *Chlorella* and *Scenedesmus* strains was required, this MTAP medium was supplemented with 0.25% glucose (w/v). The only carbon source *C. reinhardtii* can utilize heterotrophically is acetate (Sagar and Granick 1953), therefore such growth could only be accomplished in the complete TAP medium. After the pH of all media was adjusted to 6.9 by the addition of 1 M NaOH, it was sterilized by autoclaving at 140KPa (equivalent to 126^{o}C) for 20 minutes.

CULTURE CONDITIONS

Liquid cultures of the algal strains were initiated by transferring aseptically 1 ml of a suspension of the required algal strain to 20 ml of the appropriate media sterilized in 100 ml Erlenmeyer flasks, plugged with cotton wool enclosed in cheese cloth and further protected by aluminium foil caps. Similar shaped flasks were chosen to minimize variation of their internal surface area, since PCB's may bind to glass surfaces resulting in their partial removal from the media (Gresshoff *et al.* 1977).

The cultures were incubated in a shaking water bath at 20^{o}C under a bank of fluorescent lights (690 lx) with a 16 h photoperiod. All flasks were placed in random order under the light source to reduce any bias arising from the artificial lighting. When culturing in the dark was required, flasks were wrapped in aluminium foil.

The PCB used in this study was Aroclor 1242^{R} from the Monsanto Chemical Company, St. Louis, U.S.A. Aroclor 1242 is a mixture of PCB's with a mean chlorine content of 42% by weight. A stock solution of 2% (w/v) Aroclor 1242 in methanol was prepared. Serial dilutions were made from this in the appropriate sterile media to obtain the required final concentration of PCB's by adding 0.1 ml to each of the treatment flasks. Solvent controls contained 0.1 ml of methanol diluted in the same manner and normal (non solvent) controls contained

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^{R} registered trade mark

0.1 ml of additional sterile media. These additions were made approximately 1 h after the algal cultures were established. Prior to washing, all glassware was soaked overnight in methanol to removed any possible traces of PCB contamination from previous experiments.

EXPERIMENTAL DESIGN

The effect of PCB's on photosynthesis was examined by monitoring heterotrophic and photoautotrophic growth of the algae in the presence and absence of PCB's. *C. vulgaris*, *C. fusca* var. *vacuolata* and *S. obliquus* were cultured in both glucose-supplemented and minimal media in the light, and in glucose-supplemented media in the dark, with PCB treatments and appropriate controls. *C. reinhardtii* was cultured in the light only with both MTAP and TAP media and methanol controls. The test concentrations of PCB's for each algal strain was selected after consulting previous studies (Mahanty and Gresshoff 1978, Mahanty 1979). These were 200, 1, 0.5 and 4 mgl^{-1}(ppm) for *C. vulgaris*, *C. fusca* var. *vacuolata*, *S. obliquus* and *C. reinhardtii*. Growth of all cultures was assessed every 24 h for five or six days by direct cell counts in a "Neubrager" haemocytometer. All experiments were performed in triplicate at least twice.

RESULTS

Comparison of the growth patterns of all five algal strains under heterotrophic and photoautotrophic conditions show clearly a differential growth response to Aroclor 1242 at defined concentrations (Figs. 1-4). There were no noticeable differences in the growth patterns between normal controls and methanol controls. Consequently to avoid crowding on the graphs, the figures have been illustrated with only comparisons between the PCB-treatments and methanol controls. The significance of any differences between selected means maybe compared with the magnitude of the least significant differences (L.S.D.) at the 5%, 1% and 0.1% significance levels (given in the upper left corner of Figs. 1-3). These L.S.D.'s were calculated from the pooled date of all replicates for each mean (Sokal and Rohlf 1969). This was justified only after logarithmic transformation of the data to give homogenous variances as evaluated by Bartless's Test ($p = 0.1$, 0.5 and 0.3 for *C. vulgaris*, *C. fusca* var. *vacuolata* and *S. obliquus* respectively) (Sokal and Rohlf 1969).

Figs. 1-3: Mean (N = 3) growth of unicellular algae cultured heterotrophically and photoautotrophically in the presence of Aroclor 1242.

L.S.D. = Least significant difference at 5%, 1% and 0.1% levels.
Glucose supplemented media, light (○ ●) and dark (□ ■); minimal media, light (▽ ▼).
Methanol controls (○ □ ▽); Aroclor 1242 treatments (● ■ ▼).

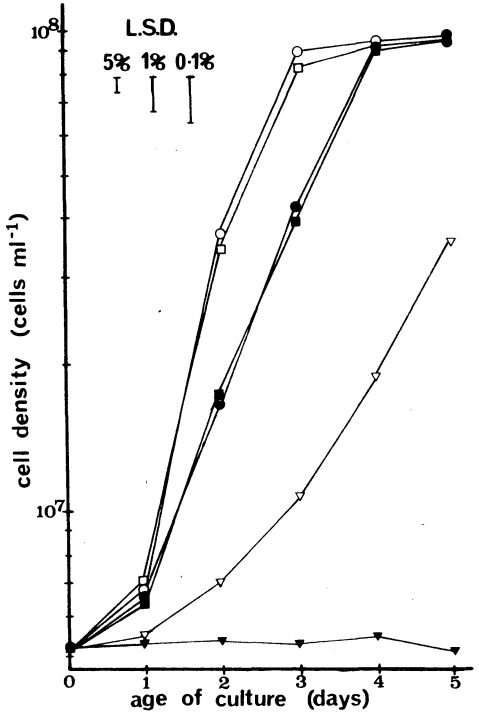

Fig. 1 *Chlorella vulgaris* (strain 211/8k) in 200 mgl^{-1}

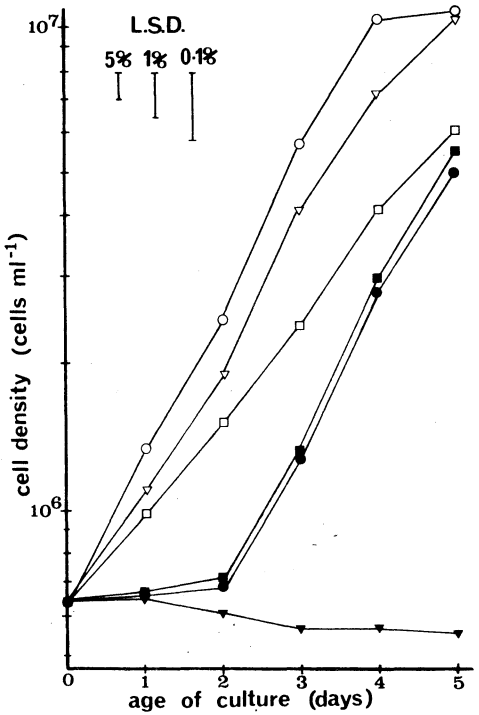

Fig. 2 *Chlorella fusca* var. *vacuolata* (strain 211/8b) in 1 mgl^{-1}

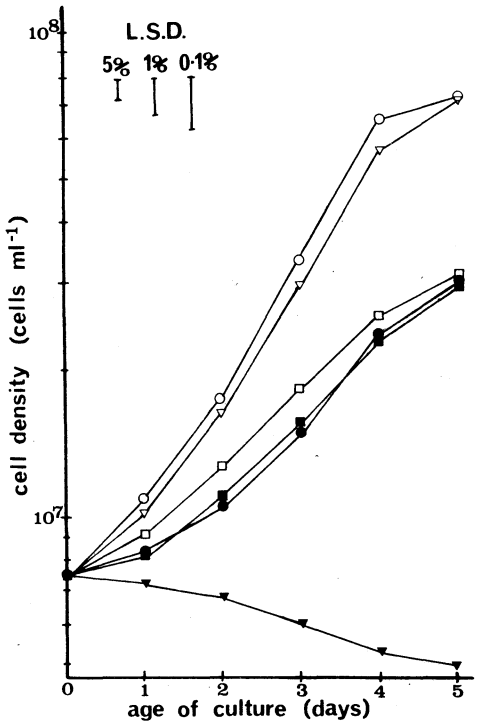

Fig. 3 *Scenedesmus obliquus* (strain 2015) in 0.5 mgl^{-1}

(a) *C. vulgaris* (strain 211/8k), (Fig. 1).

The growth of *C. vulgaris* was completely inhibited at 200 mgl^{-1} Aroclor 1242 in minimal media. In contrast growth was rapid in this media supplemented with glucose. Although the PCB treatments showed a slight lag with respect to the controls, both attained the same maximum cell denisty after four days.

(b) *C. fusca* var. *vacuolata* (strain 211/8b), (Fig. 2).

At 1 mgl^{-1} Arcolor 1242, the growth of *C. fusca* var. *vacuolata* in minimal media was completely inhibited. However after an initial lag period of two days growth in media supplemented with glucose was rapid. After five days the cumulative growth of these PCB treatments was almost equal to that of the controls.

(c) *S. obliquus* (strain 2015), (Fig. 3).

As for the previous algal strains, *S. obliquus* showed steady growth in glucose-supplemented media compared with total inhibition in minimal media when treated with 0.5 mgl^{-1} Aroclor 1242.

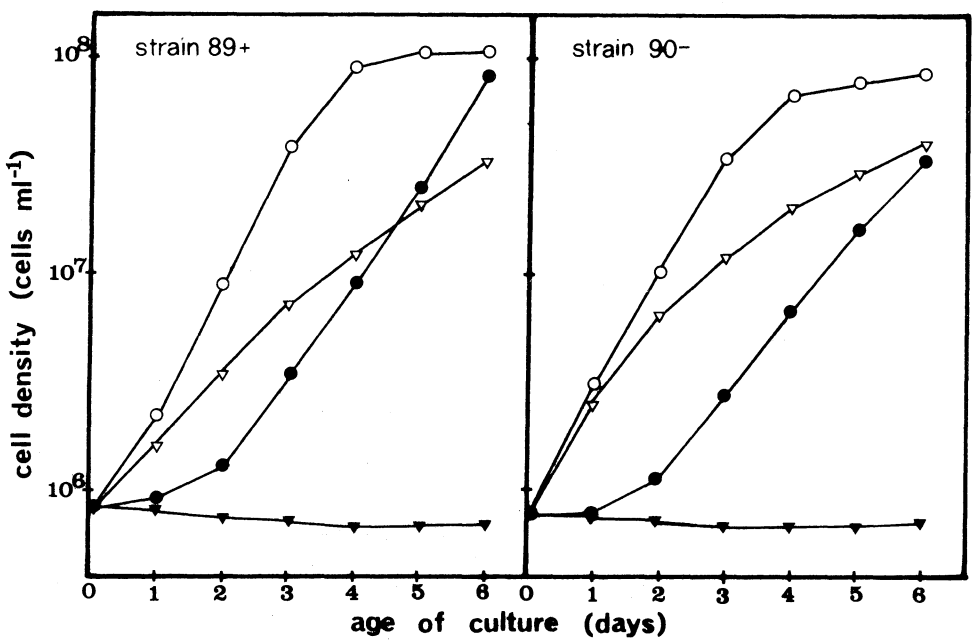

Fig. 4. Differential growth response of *Chlamydomonas reinhardtii* (strains 89+ and 90-) to 4 mgl^{-1} Aroclor 1242 when cultured in media containing acetate ( ) and minimal media ( ). Controls ( ); Aroclor treatments ( ): N = 3.

Figs. 2 and 3 show that the growth patterns of the PCB treatments in glucose-supplemented media are identical in the dark and the light; whereas the controls show that although growth in the dark is extensive, it is significantly less than that in the light. This is also apparent for *C. vulgaris* (Fig. 1), but to a much lesser extent.

(d) *C. reinhardtii* (strains 89+ and 90-), (Fig. 4).

These two strains showed complete inhibition of growth in minimal media with 4 mgl^{-1} Aroclor 1242. This is in contrast to rapid growth, after a lag period of nearly 2 days, when cultured in media containing acetate.

DISCUSSION

The results demonstrate clearly that each of the five algal strains show an increased tolerance to PCB's when a utilizable carbon source is present in their culture media. This suggests that these algae utilize the carbon source to by-pass a metabolic block induced by PCB's. Furthermore, in the presence of PCB's and glucose, the growth of the *Chlorella* strains and *S. obliquus* in the light was identical to that in the dark. The cumulative growth of these two treatments did not differ significantly from the dark-grown controls after five days. The fact that the light-grown control on glucose-supplemented media showed greater growth than each of these three treatments suggests that the algae are not photosynthesizing in the presence of PCB's, but are deriving their total nutrition heterotrophically from the supplied glucose. It is therefore proposed that PCB's have an inhibitory effect on some part of the photosynthetic mechanism of algae.

Heterotrophic growth of the five algal strains in the presence of PCB's showed a lag period of approximately two days before any growth was recorded. Similar lag periods have been noted previously for *C. reinhardtii* (Morgan 1972, Gresshoff *et al.* 1977, Mahanty and Gresshoff 1978), *C. fusca* var. *vacuolata* (Gresshoff *et al.* 1977), *Chlorella pyrenoidosa* (Hawes *et al.* 1976a) and *Rhizosolenia setigera* (Fisher and Wurster 1973). These lag periods apparently occur when phytoplankton are exposed to sub-lethal concentrations of PCB's. Despite these lag periods, growth in the presence of PCB's only occurred when carbohydrates were supplied. After five days this growth was equivalent to that of the controls.

There is much evidence both for and against the view that photosynthesis is the primary process affected by PCB's. The majority of workers employed ^{14}C- bicarbonate uptake techniques and their results show very little agreement. Some of these inconsistencies may result from variation between workers with respect to:

(1) different experimental organisms;
(2) different PCB compounds differing in toxicity;
(3) different exposure times to both PCB's and ^{14}C-bicarbonate;
(4) different concentrations of PCB's; and
(5) assorted culture conditions.

Nevertheless, many of the conflicting results can be explained by the questionable experimental design employed by some workers. Södergren (1971) showed that maximum uptake of PCB's by *Chlorella pyrenoidosa* did not occur until after 4 days exposure and that only half the maximum uptake had occurred after 12 hours. Consequently, as Sinclair *et al.* (1977) exposed *C. vulgaris* to PCB's for only 30 min. their finding of little effect on photosynthesis is to be expected; moreover, their results were expressed on a per unit of chlorophyll basis. Similarly, Biggs *et al.* (1978) expressed ^{14}C-bicarbonate uptake per chlorophyll unit, and although they measured a decrease for the first few days, they reported slight stimulations after six days. It is known that PCB's reduce chlorophyll levels in phytoplankton, even after corrections for cell density (Ewald *et al.* 1976; Powers *et al.* 1977). Therefore the use of chlorophyll level as an index for cell density in PCB treated cultures of phytoplankton may give misleading results, and the expression of ^{14}C-bicarbonate uptake per unit of chlorophyll may not give a reliable indication of any effect on photosynthesis. This was demonstrated by Powers *et al.* (1977), who showed that both chlorophyll *a* and ^{14}C-bicarbonate uptake were reduced on a per cell basis in the presence of PCB's, while no effect was apparent when ^{14}C-bicarbonate uptake was calculated per unit chlorophyll.

Opinion is divided as to whether or not ^{14}C-bicarbonate uptake on a per cell basis is affected by the presence of PCB's (see introduction). An important factor overlooked in these experiments, which may have a bearing on the apparent discrepancies, is that at higher cell densities the rate of photosynthesis per cell decreases due to internal shading within cultures (Burlew 1953, Meyers 1962, Fogg 1965). Perhaps there was reduced carbon fixation per cell due to internal shading in the controls of these experiments. This is unlikely to occur in the PCB treatments because of their lower cell density. Therefore, any reductions of ^{14}C-bicarbonate uptake per cell due to the presence of PCB's is possibly masked by similar reductions in the control through internal shading.

Powers *et al.* (1977) have studied a natural marine phytoplankton community dominated by two *Thalossiosira* species, and found that chlorophyll *a* levels and carbon fixation per cell were reduced by $10ug1^{-1}$ PCB, whereas carbon fixation per unit chlorophyll was not. They speculated that the PCB-induced inhibition of photosynthesis was due to an interference with chlorophyll synthesis, rather than with photosynthesis *per se*. The experimental design employed here allows this hypothesis to be tested. Is chlorophyll synthesis reduced in the presence of PCB's when the cells are growing heterotrophically? Likewise, it is possible to test whether the observed disruptions of internal chloroplast membranes (Glooschenko and Glooschenko 1975, Mahanty and Fineran 1976) are a direct effect of the presence of PCB's or whether they are induced by the photosynthetic block. It may not be possible to test these hypotheses for all unicellular algae, as several species undergo bleaching and chloroplast degeneration when grown heterotrophically (Hase 1975). Chlorophyll levels were not measured in this investigation, however the heterotrophically grown cultures of *C. fusca* var. *vacuolata*, *C. reinhardtii* and *S. obliquus*

appeared to be equal in intensity of "greeness" in both the controls and PCB treatments (*C. vulgaris* showed bleaching with heterotrophic growth). If PCB's directly affect chlorophyll synthesis, then some bleaching of these cultures on exposure to PCB's would be expected, especially when cell densities reached 10^7 - 10^8 cells ml^{-1} after 5 days. Consequently, we do not favour the hypothesis of Powers *et al.* (1977) but consider that the observed reductions in chlorophyll are a secondary effect of a photosynthetic blockage. Nevertheless, this still remains to be tested quantitively.

Concentration of PCB's used in this investigation are many times greater than the levels reported in natural waters. However, PCB concentrations up to 0.431 mgl^{-1} (Kpekata 1975) and 2.8 mgl^{-1} (Nadeau and Davis 1976) were recorded in rivers after recent PCB spillages. High levels of PCB's were used in this investigation because the experimental design required concentrations sufficient to completely inhibit the growth of the unicellular algae in minimal media. Phytoplankton take up and accumulate PCB's (Keil *et al.* 1971, Södergren 1971, 1973, Morgan 1972, Scura and Theilacker 1977, Harding and Phillips 1978b) and, because this uptake can occur to the same extent in living and dead cells (Urey *et al.* 1976), it is a passive process rather than an active one. Consequently, as PCB's are lipophilic (Fishbein 1972), their accumulation in phytoplankton probably involves a simple partitioning into the cellular lipids. It is therefore possible for phytoplankton to accumulate high concentrations of PCB's in their cells by continual exposure to low concentrations. In addition, Harding and Phillips (1978c) recently showed that phytoplankton rapidly take up ^{14}C-labelled PCB's from particulate matter. A high content of PCB's exists in the sediments of aquatic systems despite the presence of only trace amounts in the water (Duke *et al.* 1970, Glooschenko *et al.* 1976). The particulate matter of the sediments therefore provides an additional source from which algal cells may accumulate PCB's. The photosynthetic apparatus of unicellular algae may be also affected at PCB concentrations lower than those found in the natural environment, but in a non-lethal manner. This may result in slight reductions in the primary productivity of aquatic ecosystems.

Fisher and Wurster (1974) raised doubts about extrapolating laboratory results to the natural environment because organisms do not exist in isolation, or under ideal conditions in nature. They suggest that sub-lethal concentration of PCB's in the laboratory become growth inhibiting in natural ecosystems due to stress or suboptimal conditions. However, this investigation has shown that some phytoplankton may survive a lethal dose of PCB's in natural ecosystems by living heterotrophically through the utilization of carbohydrates. Such a mode of nutrition may be important until concentrations of biologically available PCB's have dissipated throughout the environment, resulting in their effective reduction to a lower, less inhibitory level. Carbohydrates are available for heterotrophic growth in natural ecosystems from decaying litter, plant exudates and animal excreta. It has been suggested recently (Abeliovich and Welsman 1978) that *S. obliquus* in oxidation ponds may obtain at least 15% and possibly up to 25-50% of its carbon nutrition heterotrophically.

Recently, Bryan and Olafsson (1978) found that 10 mgl^{-1} Aroclor 1242 induced a significant inhibition in the growth of *Euglena gracilis* (strain Z). At the time this appeared to contradict a previous report claiming that Aroclor 1242 at 100 mgl^{-1} had no effect on the growth of this straing (Ewald *et al.* 1976). An important difference easily overlooked when making this comparison, is that Ewald *et al.* (1976) included 1% glucose in their culture media. It is known that the strain Z of *E. gracilis* is capable of utilizing glucose heterotrophically (Pringsheim 1955, Hutner *et al.* 1956). The present investigation has demonstrated that phytoplankton are capable of tolerating higher concentrations of PCB's when their culture media are supplemented with a carbon source which they can utilize heterotrophically. Consequently the different sensitivity of *E. gracilis* (strain Z) to Aroclor 1242 reported by Ewald *et al.* (1976) and by Bryan and Olafsson (1978) is expected.

This primary inhibition of photosynthesis induced in phytoplankton by PCB's may also occur in higher plants. Uptake and translocation of PCB's by species of higher plants from both aquatic ecosystems (Moza *et al.* 1973, 1974, Walsh *et al.* 1974) and terrestrial ecosystems (Iwata *et al.* 1974, Suzuki *et al.* 1977) is known and Mahanty (1975) observed growth inhibitions of an aquatic angiosperm *(Spirodela oligorrhiza)* in the presence of PCB's.

Living systems are in a well-balanced dynamic state, and by altering one metabolic sequence another many be indirectly distrubed, resulting in further indicrect disruptions in cell physiology. These accumulate and lead to damage sufficient to seriously alter the metabolism of a cell or even kill it. The approach taken in this investigation succeeded in overcoming the total inhibition of growth by supplying unicellular algae with a carbohydrate source, thereby enabling them to survive heterotrophically. This demonstrates that the primary inhibiton induced by PCB's is associated with photosynthesis, and that other physiological responses such as reduced RNA lelvels (Keil *et al.* 1971, Mahanty and McWha 1976) and reduced chlorophyll levels (Keil *et al.* 1971, Mahanty and McWha 1976, Ewald *et al.* 1976, Powers *et al.* 1977, Biggs *et al.* 1978) are only indirect effects.

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LITERATURE CITED

ABELIOVICH, A. and WELSMAN, D. 1978. Role of heterotrophic nutrition in growth of the alga, *Scenedesmus obliquus* in high-rate oxidation ponds. *Applied and Environmental Microbiology 35:* 32-37.

ANONYMOUS. 1966. Report of a new chemical hazard. *New Scientist 32:* 612

BIGGS, D.C., ROWLAND, R.G., O'CONNORS, H.B. Jr., POWERS, C.D. and WURSTER, C.F. 1978. A comparison of the effects of Chlordane and PCB on the growth, photosynthesis and cell size of estuarine phytoplankton. *Environmental Pollution 15:* 253-263.

BOWES, G. W. 1972. Uptake and metabolism of DDT by marine phytoplankton and its effect on growth and chloroplast electron transport. *Plant Physiology 49:* 172-176.

BOWES, G.W. and GEE, R.W. 1971. Inhibition of photosynthetic electron transport by DDT and DDE. *Journal of Bioenergetics 2:* 47-60.

BRYAN, A.M. and OLAFSSON, P.G. 1978. The effect of Polychlorobiphenyls (Aroclor 1242) on bicarbonate ^{14}-C uptake by *Euglena gracilis*. *Bulletin of Environmental Contamination and Toxicology 19:* 374-381.

BURLEW, J.S. 1953. (Ed). *Algal culture from laboratory to pilot plant.* Carnegie Institution of Washington, publication number 600, 357 pp.

COLE, D. R. and PLAPP, F.W. Jr. 1974. Inhibition of growth and photosynthesis in *Chlorella pyrenoidosa* by a Polychlorinated Biphenyl and several insecticides. *Environmental Entomology 3:* 217-220.

CRAIGIE, J. S. and HUTZINGER, O. 1975. Effects of commercial chlorinated hydrocarbons and specific chlorobiphenyls on the growth of seven species of marine phytoplankton. *Chemosphere 4:* 139-144.

DUKE, T.W., LOWE, J.I. and WILSON, A.J. Jr 1970. A PCB (Aroclor 1254) in the water, sediment and biota of Escambia Bay, Florida. *Bulletin of Environmental Contamination and Toxicology 5:* 171-180.

EWALD, W.G., FRENCH, J. E. and CHAMP, M.A. 1976. Toxicity of Polychlorinated Biphenyls (PCB's) to *Euglena gracilis:* Cell population growth, carbon fixation, chlorophyll level, oxygen consumption and protein and nucleic acid synthesis. *Bulletin of Environmental Contamination and Toxicology 16:* 71-80.

FISHBEIN, L. 1972. Chromatographic and biological aspects of Polychlorinated Biphenyls. *Journal of Chromatography 68:* 345-426.

FISHER, N.S. 1975. Chlorinated hydrocarbon pollutants and photosynthesis of marine phytoplankton: A reassessment. *Science 189:* 463-464.

FISHER, N. S. 1977. On the differential sensitivity of estuarine and open-ocean diatoms to exotic chemical stress. *The American Naturalist 111:* 871-895.

FISHER, N. S., CARPENTER, E.J., REMSON, C.C. and WURSTER, C.F. 1974. Effects of PCB on interspecific competition in natural and gnotobiotic phytoplankton communities in continuous and batch cultures. *Microbial Ecology 1:* 39-50.

FISHER, N.S., GRAHAM, L.B., CARPENTER, E.J. and WURSTER, C.F. 1973. Geographic differences in phytoplankton sensitivity to PCB's. *Nature 241:* 548-549.

FISHER, N.S., GUILLARD, R.R.L. and WURSTER, C.F. 1976. Effects of a chlorinated hydrocarbon pollutant on the growth kinetics of a marine diatom. pp. 305-317. *In:* Canale, R.P. (Ed.) *Modelling Biochemical Processes in Aquatic Ecosystems.* Ann Arbor Science Publishers, Ann Arbor, Michiagan. 389 pp.

FISHER, N.S. and WURSTER, C.F. 1973. Individual and combined effects of temperature and Polychlorinated Biphenyls on the growth of three species of phytoplankton. *Environmental Pollution 5:* 205-212.

FISHER, N.S. and WURSTER, C.F. 1974. Impact of pollutants on plankton communities. *Environmental Conservation 1:* 189-190.

FOGG, G. E. 1965. *Algal Cultures and Phytoplankton Ecology.* The Athone Press, University of London. 126pp.

GIAM, C.S., WONG, M.K., HANKS, A.R., SACKETT, W.M. and RICHARDSON, R.L. 1973. Chlorinated hydrocarbons in plankton from the Gulf of Mexico and Northern Caribbean. *Bulletin of Environmental Contamination and Toxicology 9:* 376-382.

GLOOSCHENKO, V. and GLOOSCHENKO, W. 1975. Effect of Polychlorinated Biphynyl compounds on growth of Great Lakes phytoplankton. *Canadian Journal of Botany 53:* 653-659.

GLOOSCHENKO, W.A,, STRACHAN, W. M. J. and SAMPSON, R.C.J. 1976. Distribution of pesticides and Polychlorinated Biphenyls in water, sediments and seston of the Upper Great Lakes - 1974. *Pesticides Monitoring Journal 10:* 61-67.

GRESSHOFF, P.M. 1976. Cultures of *Chlamydomonas reinhardi* protoplasts in defined media. *Australian Journal of Plant Physiology 3:* 457-464.

GRESSHOFF, P.M., MAHANTY, H.K. and GÄRTNER, E. 1977. Fate of Polychlorinated Biphenyl (Aroclor 1242) in an experimental study and its significance to the natural environment. *Bulletin of Environmental Contamination and Toxicology 17:* 686-691.

HARDING, L.W. Jr. 1976. Polychlorinated Biphenyl inhibition of marine phytoplankton photosynthesis in the Northern Adriatic Sea. *Bulletin of Environmental Contamination and Toxicology 16:* 559-566.

HARDING, L.W. Jr and PHILLIPS, J.H. Jr. 1978a. Polychlorinated Biphenyl (PCB) effects on marine phytoplankton photosynthesis and cell division. *Marine Biology 49:* 93-101.

HARDING, L.W. Jr and PHILLIPS, J. H. Jr. 1978b. Polychlorinated Biphenyl (PCB) uptake by marine phytoplankton. *Marine Biology 49:* 103-111.

HARDING, L.W. Jr. and PHILLIPS, J.H. Jr. 1978c. Polychlorinated Biphenyls: Transfer from microparticulates to marine phytoplankton and the effects on photosynthesis. *Science 202:* 1189-1192.

HARVEY, G.R., MIKLAS, H.P., BOWEN, V.T. and STEINHAUER, W. G. 1973. Observations on the distribution of Chlorinated Hydrocarbons in the Atlantic Ocean organisms. *Journal of Marine Research 32:* 103-118.

HASE, E. 1975. Recent progress in the physiology of *Chlorella*. pp.171-180. *In:* Tokida, J. and Hirose, H. (Eds). *Advances in Phycology in Japan.* Dr. W. Junk Publishers, The Hague. 355pp.

HAWES, M. L., KRICHER, J.C. and UREY, J.C. 1976a. The effects of various Aroclor fractions on the population growth of *Chlorella pyrenoidosa. Bulletin of Environmental Contamination and Toxicology 15:* 14-18.

HAWES, M.L., KRICHER, J.C. and UREY, J.C. 1976b. The effects of various Aroclor fractions on the productivity of *Chlorella pyrenoidosa. Bulletin of Environmental Contamination and Toxicology 15:* 588-590.

HUNTER, S.H., BACH, M.K. and ROSS, G.I.M. 1956. A sugar containing basal medium for vitamin B_{12}-assay with *Euglena:* Application to body fluids. *The Journal of Protozoology 3:* 101-112.

IWATA, Y., GUNTHER, F. A. and WESTLAKE, W.E. 1974. Uptake of a Polychlorinated Biphenyl (Aroclor 1254) from soil by Carrots under field conditions. *Bulletin of Environmental Contamination and Toxicology 11:* 523-528.

JENSON, S., RENBERG, L. and OLSSON, M. 1972. PCB contamination from boat bottom paint and levels of PCB in plankton outside a polluted area. *Nature 240:* 358-360.

KEIL, J.E., PRIESTER, L.E. and SANDIFER, S.H. 1971. Polychlorinated Biphenyl (Aroclor 1242): Effects of uptake on growth, nucleic acids and chlorophyll of a marine diatom. *Bulletin of Environmental Contamination and Toxicology 6:* 156-159.

KPEKATA, A.E. 1975. Polychlorinated Biphenyls (PCB's) in the Rivers Avon and Frome. *Bulletin of Environmental Contamination and Toxicology 14:* 687-691.

KRICHER, J.C. and BAYER, C. L. 1977. Depression of primary productivity by Aroclor 1232 in an interspecific lentic algal assembledge. *Bulletin of Environmental Contamination and Toxicology 18:* 14-17.

LARSSON, C.M. and TILLBERG, J.E. 1975. Effects of the commercial Polychlorinated Biphenyl mixture Aroclor 1242 on growth, viability, phosphate uptake, respiration and oxygen evolution in *Scenedesmus. Physiologia Plantarum 33:* 256-260.

LAWLER, P.D. and RODGERS, L.J. 1967. Effect of DDT on photosynthesis in certain varieties of barley. *Nature 215:* 1515-1516.

LAWLER, P.D. and RODGERS, L.J. 1968. Inhibition of photophosphorylation by 1;1;1-Trichloro-2;2-bis-(*p*-chlorophenyl)-ethane (DDT). *Biochemical Journal 110:* 381-383.

LINKO, R.R., RANTAMÄKI, P. and URPO, K. 1974. PCB residues in plankton and sediment in the southwestern coast of Finland. *Bulletin of Environmental Contamination and Toxicology 12:* 733-738.

LUARD, E.J. 1973. Sensitivity of *Dunaliella* and *Scenedesmus* (Chlorophyceae) to Chlorinated Hydrocarbons. *Phycologia 12:* 29-33.

MAHANTY, H.K. 1975. A study on the effects of Polychlorinated Biphyenyl (Aroclor 1242) on an aquatic plant - *Spirodela oligorhiza* (Kurz) Hegelm. *Bulletin of Environmental Contamination and Toxicology 14:* 558-561.

MAHANTY, H.K. 1979. Kanamycin-resistant *Scenedesmus obliquus* showing simultaneous resistance to other antibiotics and Polychlorinated Biphenyls. *Nature 277:* 562-563,

MAHANTY, H.K. and FINERAN, B.A. 1976. Effects of a Polychlorinated Biphenyl (Aroclor 1242) on the ultrastructure of frond cells in the aquatic plant *Spirodela oligorrhiza* (Kurz) Hegelm. *New Zealand Journal of Botany 14:* 13-18.

MAHANTY, H.K. and GRESSHOFF, P.M. 1978. Influence of Polychlorinated Biphenyls (PCB's) on growth of freshwater algae. *Botanical Gazette 139:* 202-206,

MAHANTY, H.K. and McWHA, J.A. 1976. Sensitivity of *Spirodela oligorrhiza* (Kurz) Hegelm. to a Polychlorinated Biphenyl (Aroclor 1242). *New Zealand Journal of Botany 14:* 9-12.

MEYERS, J. 1962. Laboratory cultures. pp.603-615 *In* Lewin, R.A. (Ed). *Physiology and Biochemistry of Algae.* Academic Press, New York. 929 pp.

MOORE, S.A. and HARRISS, R.C. 1972. Effects of Polychlorinated Biphenyls on marine phytoplankton communities. *Nature 240:* 356-357.

MOORE, S.A. and HARRISS, R.C. 1974. Differential sensitivity to Polychlorinated Biphenyls by phytoplankton. *Marine Pollution Bulletin 5:* 174-176.

MORGAN, J.R. 1972. Effects of Aroclor 1242 (a Polychlorinated Biphenyl) and and DDT on cultures of an alga, protozoan, daphnid, ostracod and guppy. *Bulletin of Environmental Contamination and Toxicology 8:* 129-137.

MOSSER, J.L., FISHER, N.S. and WURSTER, C.F. 1972. Polychlorinated Biphenyl's and DDT alter species composition in mixed cultures of algae. *Science 176:* 533-535.

MOSSER,J.L., TENG, T.C., WALTHER, W.G. and WURSTER, C.F. 1974. Interactions of PCB's, DDT and DDE in a marine diatom. *Bulletin of Environmental Contamination and Toxicology 12:* 665-668.

MOZA, P., WEISBERBER, I., KLEIN, W. and KORTE, F. 1973. Beigrage zur okologischen chemie LXIII- Verteilung und metabolismus von 2,2'-Dichlorobiphenyl - ^{14}C in der höheren sumpfpflanze, *Veronica beccabunga. Chemosphere 2:* 217-222.

MOZA, P., WIESBERBER, I., KLEIN, W. and KORTE, F. 1974. Metabolism of 2,2'-Dichlorobiphenyl -^{14}C in two plant-water-soil systems. *Bulletin of Environmental Contamination and Toxicology 12:* 541-546.

NADEAU, R.J. and DAVIS, R.A. 1976. Polychlorinated Biphenyl's in the Hudson River (Hudson Falls -Ford Edward, New York State). *Bulletin of Environmental Contamination and Toxicology 16:* 436-444.

O'CONNORS, H.B. Jr., WURSTER, C.F., POWERS, C.D., BIGGS, D.C. and ROWLAND,R.G. 1978. Polychlorinated Biphenyls may alter marine trophic pathways by reducing phytoplankton size and production. *Science 201:* 737-739.

POWERS, C.D., ROWLAND, R.G., O'CONNORS, H.B. Jr and WURSTER, C.F. 1977. Response to Polychlorinated Biphenyls of marine phytoplankton isolates cultured under natural conditons. *Applied and Environmental Microbology 34:* 760-764.

PRINGSHEIM, E.G. 1955. Klein mitteilunge über flagellaten und algen II

Euglena gracilis var. *saccharophila* n. var., und eine vereinfachte nahrlosung zur vitamin B_{12} - bestimmung. *Archiv für Mikrobiologie 21:* 414-419.

SAGAR, R. and GRANICK, S. 1953. Nutritional studies with *Chlamydomonas reinhardi*. *Annals of the New York Academy of Science 56:* 831-838.

SÄRKKÄ, J., HATTULA, M., JANATUINEN, J. and PAASIVIRTA, J. 1978. Mercury and chlorinated hydrocarbons in plankton of Lake Päijänne, Finland. *Environmental Pollution 16:* 41-49,

SCURA, E.D. and THEILACKER, G.H. 1977. Transfer of the chlorinated hydrocarbon, PCB, in a laboratory marine food chain. *Marine Biology 40:* 317-325.

SINCLAIR, J., GARLAND, S., ARNASON, T., HOPE, P. and GRANVILLE, M. 1977. Polychlorinated Biphenyls and their effects on photosynthesis and respiration. *Canadian Journal of Botany 55:* 2679-2684.

SÖDERGREN, A. 1971. Accumulation and distribution of chlorinated hydrocarbons in cultures of *Chlorella pyrenoidosa* (Chlorophyceae). *Oikos 22:* 215-220.

SÖDERGREN, A. 1973. Transport, distribution and degradation of chlorinated hydrocarbons residues in aquatic model ecosystem. *Oikos 24:* 30-41.

SOKAL, R.R. and ROHLF, F.J. 1969. *Biometry*. W.H. Freeman, San Francisco. 776 pp.

SUZUKI, M., AIZAWA, N., OKANO, G. and TAKAHASHI, T. 1977. Translocation of Polychlorobiphenyls in soil into plants: A study by a method of culture of soybean sprouts. *Archives of Environmental Contamination and Toxicology 5:* 343-352.

UREY, J.C., KIRCHER, J.C. and BOYLAN, J.M. 1976. Bioconcentration of four pure PCB isomers by *Chlorella pyrenoidosa*. *Bulletin of Environmental Contamination and Toxicology 5:* 343-352.

WALSH, G.E. HOLLISTER, T.A. and FORESTER, J. 1974. Translocation of four organochlorine compounds by Red Mangrove (*Rhizophora mangle* L.) seedlings. *Bulletin of Environmental Contamination and Toxicology 12:* 129-135.

WARE, D.M. and ADDISON, R.F. 1973. PCB residues in plankton from the gulf of St. Lawrence. *Nature 246:* 519-521.

WOOD, E.J.F. 1965. *Marine Microbial Ecology*. Chapman and Hall, London. 243 pp.